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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/017,445	12/14/2001	Susan Rigby	BP0003-US	6377
23544	7590	03/25/2003		
BRIAN D. GILDEA APPLIED BIOSYSTEMS 15 DEANGELO DRIVE BEDFORD, MA 01730			EXAMINER	SAKELARIS, SALLY A

ART UNIT	PAPER NUMBER
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1634

DATE MAILED: 03/25/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/017,445	RIGBY ET AL.	
	Examiner	Art Unit	
	Sally A Sakelaris	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) Responsive to communication(s) filed on 14 December 2000.
 2a) This action is **FINAL**. 2b) This action is non-final.
 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) Claim(s) 1-21 is/are pending in the application.
 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
 5) Claim(s) _____ is/are allowed.
 6) Claim(s) 1-21 is/are rejected.
 7) Claim(s) _____ is/are objected to.
 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) The specification is objected to by the Examiner.
 10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 11) The proposed drawing correction filed on _____ is: a) approved b) disapproved by the Examiner.
 If approved, corrected drawings are required in reply to this Office action.
 12) The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
 * See the attached detailed Office action for a list of the certified copies not received.

14) Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
 a) The translation of the foreign language provisional application has been received.
 15) Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)	4) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s). _____
2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152)
3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449) Paper No(s) <u>31112002</u> <u>31132003</u>	6) <input type="checkbox"/> Other: _____

DETAILED ACTION

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged. The present application's claim to benefit of the provisional application, 60/256,140 filed 12/15/2000 is granted.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1. Claims 1, 2, 3, 4, 9, 10, 11, 12, 14, 16, and 18 are rejected under 35 U.S.C. 102(b) as being anticipated by Yurov et al. Human Genetics, 1996.

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).

b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);

c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg.392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the cheek and **fixed immediately** with methanol/acetic fixative”(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

2. Claims 2 and 5 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yurov et al. in view of Drobniowski et al. (Journal of Clinical Microbiology, Jan. 2000).

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).

b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);

c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg.392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the cheek and **fixed immediately** with methanol/acetic fixative(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Yurov et al. do not teach a method of detection wherein the organisms, cells or both are collected from a growth medium consisting of broth or agar.

However, Drobniowski et al. teach a method of detection wherein the organisms, cells or both are collected from a growth medium consisting of broth or agar(Pg. 444). Drobniowski et

al. teach the differentiation of two liquid cultures by using Peptide Nucleic Acid(PNA)-fluorescence in situ hybridization probes. The medium in which their mycobacterial cultures grew was based on the "Middlebrook 7H9 broth"(Pg. 444, bottom right). The reference teaches a rapid identification system for mycobacterial species in clinical specimens whose cost is not as high as that of current specialized analytical equipment.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the hybridization method of Yurov et al. so as to have included a method wherein the organisms, cells or both are collected from a growth medium for the expected benefit of providing an additional means for furthered detection of both based bacterial cultures. Therefore, combining the teachings of Yurov et al. in view of Drobniowski et al. would have been obvious at the time the invention was made.

3. Claims 6 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yurov et al. in view of Braissant et al. (Biochemica, 1998).

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).

b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);

c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that

produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg.392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the cheek and **fixed immediately** with methanol/acetic fixative(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are

determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Yurov et al. do not teach the above method wherein a blocking agent that is casein is present during the operation of step (c).

However, Braissant et al. teach a method of in situ hybridization wherein a blocking agent that is casein is present during the operation of step (c)(ie hybridization)(Pgs. 12 and 15). The references teaches that, “finally, blocking reagent treatment during hybridization” took place. As well as that “in the signal visualization step we[they] chose casein (Boehringer Mannheim Blocking Reagent) as a blocker” during their hybridization step. The reference teaches that they have “developed a simplified and efficient protocol for non-radioactive in situ hybridization experiments, . . . the sensitivity of which allows the expression of genes to be studied with transcript levels ranging from very low to high”(Pg 15).

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the in situ hybridization method of Yurov et al. so as to have included casein as a blocking reagent in the hybridization step for the expected benefit of providing a simplified and efficient protocol for additional means for a non-radioactive in situ hybridization experiments. Therefore, combining the teachings of Yurov et al. in view of Braissant et al. would have been obvious at the time the invention was made.

4. Claims 2, 4, 8, and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yurov et al. in view of Bresser et al. (US Patent 5,225,326).

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).

b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);

c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg.392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the cheek and **fixed immediately** with methanol/acetic fixative(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-

labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Yurov et al. do not teach the above method wherein the organisms, cells or both are collected from a growth medium that has not been completely separated from the sample of organisms, cells or both, nor do they teach a method wherein steps (b) and (c) are performed simultaneously nor do they teach a method for determining organisms, cells or both, said method comprising:

a). treating a sample of fixed cells, organisms or both, that have been grown in a medium, with one or more detectable molecular probes, under suitable hybridization conditions, in a way that produces stained organisms, cells or both stained organisms and cells; and

b). determining the stained cells, organism or both the stained organisms and cells; wherein said assay does not require that the medium be removed or separated from the organisms, cells or both the organisms and cells.

However, Bresser et al. teach the above method wherein the organisms, cells or both are collected from a growth medium(Col. 7, lines 34-41) that has not been completely separated from the sample of organisms, cells or both, a method wherein steps (b) and (c) are performed

simultaneously(Col.3), and a method for determining organisms, cells or both, said method comprising:

a). treating a sample of fixed cells, organisms or both, that have been grown in a medium, with one or more detectable molecular probes, under suitable hybridization conditions, in a way that produces stained organisms, cells or both stained organisms and cells; and

b). determining the stained cells, organism or both the stained organisms and cells;

wherein said assay does not require that the medium be removed or separated from the organisms, cells or both the organisms and cells(Summary of Invention, Col.2).

Bresser et al. teach that it is “an object of the present invention to provide an *in situ* hybridization procedure that could be carried out on cells in suspension” as they intend to provide a fast and sensitive *in situ* hybridization procedure capable of detecting more than one target molecule in an individual cell and eliminate the need for immobilization of cells or tissues onto a solid support before analysis(Col.2). Furthermore, Bresser et al teaches a means of carrying out the fixation, prehybridization, hybridization and detection steps normally associated with *in situ* hybridization procedures all in one step(Col. 3, lines 55-60). As a result, the fixation is accomplished in the same solution and along with the hybridization reaction. Additionally, the reference teaches the specimens to be isolated in the one-step procedure as cell suspensions that contain living or dead cells(Col. 7 lines 34-42).

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the *in situ* hybridization method of Yurov et al. so as to have included cells collected from a growth medium, that was not completely separated from the sample cells, and a method wherein steps (b) and (c) are performed

simultaneously(Col.3), and a method for determining organisms, cells or both, said method comprising:

a). treating a sample of fixed cells, organisms or both, that have been grown in a medium, with one or more detectable molecular probes, under suitable hybridization conditions, in a way that produces stained organisms, cells or both stained organisms and cells; and

b). determining the stained cells, organism or both the stained organisms and cells;

wherein said assay does not require that the medium be removed or separated from the organisms, cells for the expected benefit of providing a fast and sensitive in situ hybridization procedure. Therefore, combining the teachings of Yurov et al. in view of Bresser et al. would have been obvious at the time the invention was made.

5. Claims 13, 19, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yurov et al. in view of Ortiz et al. (Molecular and Cellular Probes, 1998).

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).

b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);

c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg. 392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the cheek and **fixed immediately** with methanol/acetic fixative(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Yurov et al. do not teach the above method wherein the molecular probe is a self-indicating molecular probe selected from the group consisting of a linear beacon, a nucleic acid or PNA molecular beacon and an intercalating beacon, or that the molecular probe is a non-nucleic acid probe which is a peptide nucleic acid.

However, Ortiz et al. teach a method of fluorescent nucleic acid detection wherein the molecular probe is a self-indicating molecular probe selected from the group consisting of a linear beacon, a nucleic acid or PNA molecular beacon and an intercalating beacon, or that the molecular probe is a non-nucleic acid probe which is a peptide nucleic acid(Pg. 225). The reference teaches that the improved hybridization stability of PNA over DNA made possible direct detection of double stranded DNA. The reference further teaches the improvement made to fluorescent dye systems, “a variety of high quantum yield fluorescent compounds, including fluorescein and other dyes, can be effectively quenched by DABCYL when employed in the molecular beacon format”(Pg. 225) The reference furthers the teachings of Tyagi and Kramer which included that molecular beacons combined increased specificity and a very efficient quenching system, to include the use of PNA probes which are able to form highly specific and extraordinarily-stable duplexes with complementary DNA sequences(Pg. 219).

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the in nucleic acid hybridization method of Yurov et al. so as to have included the PNA molecular beacon of Ortiz et al. for the expected benefit of forming highly specific and extraordinarily-stable duplexes with complementary DNA sequences hybridization experiments. Therefore, combining the teachings of Yurov et al. in view of Ortiz et al. would have been obvious at the time the invention was made.

6. Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yurov et al. in view of Iris et al. (US Patent 6,403,309 B1).

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).

b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);

c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg.392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the

cheek and **fixed immediately** with methanol/acetic fixative(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Yurov et al. do not teach a method of detection wherein the method further comprises:
e). adding a quencher labeled oligomer before the determination is made to thereby form a complex between the excess molecular probe and the quencher labeled oligomer.

However, Iris et al. do teach a method of detection wherein the method further comprises:
e). adding a quencher labeled oligomer before the determination is made to thereby form a complex between the excess molecular probe and the quencher labeled oligomer(Col. 16, lines 35-54). The reference teaches that to reduce the un-hybridized probe's contribution to the signal in the final product, an “anti-probe” is used to eliminate the fluorescent signal from the excess probe. The anti-probe comprises an oligonucleotide complementary to the probe that has two fluorescent quenchers matched to the fluorophores present on the probe of interest. The

reference adds that the “anti-probe” is added and annealed to the reaction in order to “mop-up” the fluorescence from the excess probe.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the hybridization method of Yurov et al. so as to have included the “anti-probe” of Iris et al. for the expected benefit of being able to “mop-up” the excess probe in the reaction prior to the determination step to decrease background fluorescence. Therefore, combining the teachings of Yurov et al. in view of Iris et al. would have been obvious at the time the invention was made.

7. Claims 11, 12, and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yurov et al. in view of Hyldig-Nielsen et al. (US Published Patent Application US 2001/0010910 A1).

Yurov et al. teach a method for the analysis of organisms, cells or both organisms and cells; said method comprising:

- a). collecting a sample of organisms or cells(materials and methods(m&m), Pg 391, left column).
- b). adding one or more fixative agents to the samples to thereby fix the organisms, cells or both(m&m, Pg. 391, left column);
- c).treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with molecular probe in a way that produces detectable or independently detectable organisms, cells or both(m&m, Pg. 391, right column) and;

d). determining one or more of the detectable organisms or cells in the sample(391); wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination(m&m, Pg 391 on bottom right and Pg.392 on top left).

Yurov et al. further teach a method wherein the organisms, cells or both are collected from a growth medium, such as from phytohemagglutinin-stimulated blood lymphocytes as well as from a sample that has not been treated for growth, such as from uncultured aminocyte cells or from frozen samples of colorectal tumors(m&m, Pg 391). Yurov et al teaches this same method wherein the growth medium is not completely separated from the sample of organisms, cells or both in teaching “samples of buccal smears were collected from scraping of the inside of the cheek and **fixed immediately** with methanol/acetic fixative(m&m, Pg. 391). Yurov et al. teach the method wherein steps (b) and (c) supra are performed sequentially in that order(m&m, Pg 391). Furthermore, Yurov et al. teach the above method wherein two or more detection complexes or independently detectable, fluorophore-labeled and independently detectable-labeled, probes are used in the method for the multiplex analysis of two or more **different types** of organisms or cells in the sample by teaching a method using a “battery of alphoid DNA probes...labeled by Cy3-dCTP, FluorX-dCTP, and Cy5 and the subsequent analysis of metaphase chromosomes and interphase nuclei in cytological preparations of lymphocytes, aminocytes, tumor cells, spermatozoa, and buccal epithelium(See text Pg. 392 and Fig.1 a-g). Lastly, the reference teaches the above method wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer(m&m, Pg.392 left side).

Yurov et al. do not teach the above method wherein the fluorescence in situ hybridization is a multiplex assay or a method of in situ hybridization wherein one or more blocking probes are present during the operation of step (c).

However, Hyldig-Nielsen et al. teach a method of fluorescence in situ hybridization wherein two or more independently detectable molecular probes are used in the method for the multiplex analysis of two or more different types of organisms or cells in the sample, and wherein the two or more independently detectable molecular probes are labeled with independently detectable fluorophores, and wherein one or more blocking probes are present during the operation of step (c)(Page 4,[0046, 0048, 0051, and 0057]. The reference teaches a multiplex assay in which numerous conditions of interest are simultaneously examined. “The teaching of the ability to differentiate between and/or quantitate each of the independently detectable moieties provides the means to multiplex a hybridization assay because the data which correlates with the hybridization of each of the distinctly labeled probe to a particular target sequence can be correlated with the presence, absence or quantity of each organism sought to be detected in the sample. Consequently, the multiplex assays of the present reference, may be used to simultaneously detect the presence, absence or quantity of two or more organisms in the same sample and in the same assay”([0051]). In addition, the reference teaches that blocking probes may also be used in the hybridization step as a means to improve discrimination beyond the limits possible by mere optimization of stringency factors([0057]).

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the in situ hybridization method of Yurov et al. so as to have included the multiplex assay and blocking probes of Hyldig-Nielsen et al. for the

expected benefit of being able to simultaneously detect the presence, absence or quantity of two or more organisms in the same sample and in the same assay and improve discrimination. Therefore, combining the teachings of Yurov et al. in view of Hyldig-Nielsen et al. would have been obvious at the time the invention was made.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 3 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. Claim 3 is indefinite over the recitation of “treated for growth.” It is unclear if the applicant intended for the treated for growth to mean: a sample containing alive cells, a sample that included a carbon source for nourishment, a sample that had an antibiotic added to it to stop the growth of contaminating bacteria, etc. Applicant should amend the claims to clarify their intended meaning of a sample that has not been “treated for growth.”

B. Claim 21 is indefinite over the recitation of “wherein aid assay does not require that the medium be removed” because it is not clear whether this means that the step may occur but is not essential or if this means that the method of determining organisms or cells does not include a step of removing or separating the medium. Applicant should amend the claims to clarify their requirement for removing or separating the media from the organisms cells or both.

C. Claim 21 recites the limitation "said assay" in line seven. There is insufficient antecedent basis for this limitation in the claim.

Any inquiry concerning this communication or earlier communication from the examiner should be directed to Sally Sakelaris whose telephone number is (703) 306-0284. The examiner can normally be reached on Monday-Thursday from 7:30AM-5:00PM and Friday from 1:00PM-5:00PM.

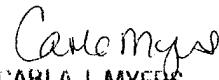
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W.Gary Jones, can be reached on (703)308-1152. The fax number for the Technology Center is (703)305-3014 or (703)305-4242.

Any inquiry of a general nature or relating to the status of this application should be directed to Chantae Dessau whose telephone number is (703)605-1237.

Sally Sakelaris



3/20/2003


CARLA J. MYERS
PRIMARY EXAMINER